

REPRINTED FROM:



WORLD CONGRESS ON IN VITRO FERTILIZATION AND ASSISTED REPRODUCTION

Vienna (Austria), April 3 - 7, 1995

Editors

A. ABURUMIEH, E. BERNAT, G. DOHR,
W. FEICHTINGER, F. FISCHL,
J. HUBER, E. MÜLLER, S. SZALAY,
W. URDL and H. ZECH

MONDUZZI EDITORE

INTERNATIONAL PROCEEDINGS DIVISION

IXA

An RIA by Amersham with a low coefficient of variation in the low serum P range associated with better pregnancy rate (PR) prognosis with IVF-ET (1)

L. HOOVER, A. O'SHAUGHNESSY, A. NAZARI
and J.H. CHECK

UMDNJ, Robert Wood Johnson Med. School at Camden (USA)

SUMMARY

Previous studies using a radioimmunoassay (RIA) found a correlation of higher pregnancy rates (PRs) with lower serum progesterone (P) levels when human chorionic gonadotropin (hCG) is given in in vitro fertilization (IVF) cycles when luteal phase leuprolide acetate/human menopausal gonadotropin was used for the controlled ovarian hyperstimulation (COH) regimen. Our study attempted to corroborate these findings using a different RIA for P when the same COH regimen was administered. PRs were significantly higher when P was $\leq 1\text{ng/mL}$ at the time of hCG (43.2%) vs the groups where the P level ranged from 1.1 to 2ng/mL (15.8%). Viable PRs were also significantly higher in the lower P group. In contrast to the previous data with the DPC assay, no differences were seen with P $< 0.5\text{ng/mL}$ (36.4%) vs 0.5 to 1ng/mL (44.6%).

INTRODUCTION

Several recent publications have suggested an association with lowered PRs with even subtle increases in serum P without overt premature luteinization occurring at the time hCG is administered in IVF

cycles, even when using gonadotropin releasing hormone agonist (GnRHa) down regulation, followed by human menopausal gonadotropin (hMG) stimulation for the COH regimen. Schoolcraft et al. found much higher PRs in patients whose serum P was $<0.5\text{ng/mL}$ at the time of hCG; these data were confirmed by Silverberg et al. (2,3).

Both of these studies used a commercial RIA kit for measuring serum P (Diagnostic Products Corporation, Los Angeles, CA); the inter and intra-assay coefficients of variation (CV) were listed at 11.8% and 8.9%. We also use a commercial RIA kit for P measurement (Amersham-Amerlex, Arlington Heights, IL) in our laboratory and have found the inter and intra-assay CV to be 9.1% and 11.9%, respectively.

The study presented herein evaluated whether a low serum P could also discern those patients with the highest PRs when the Amersham RIA was used to measure serum P. It is important to evaluate each RIA kit separately, because for some assays the CV may be larger and thus different conclusions may be reached. For example, Edelstein et al. found no prognosticating value of the higher serum P level at the time of hCG on subsequent PRs, but their assay (Pantex, Santa Monica, CA) had a 34% CV.

MATERIALS AND METHODS

The subjects of the study were 119 consecutive patients having in vitro fertilization-embryo transfer (IVF-ET). Each patient was started on leuprolide acetate (LA) 1mg/day beginning in mid-luteal phase. The dosage was decreased to 0.5mg/day after ten days if P was adequately suppressed to $<1.5\text{ng/mL}$ and estradiol (E_2) to $<50\text{pg/mL}$. Human menopausal gonadotropins (hMG), 300IU/d (4 ampules) administered intramuscularly (IM) were started on day 11 and reduced to 225IU/d (3 ampules) after four days. Further changes were made as needed according to the results of serum E_2 and the number and size of the follicles, as determined by pelvic sonography using a transvaginal transducer. A single IM injection of 10,000 units of hCG was given when at least two follicles attained an average diameter of 18mm and the serum E_2 was at least 800pg/mL.

Patients were divided into five groups according to the serum P concentration at the time of hCG administration: grp 1 - P $<.5\text{ng/mL}$; grp 2 - P 0.5 to $\leq 1\text{ng/mL}$; grp 3 - P 1.1 to 1.5ng/mL; grp 4 - P 1.6 to 2.0ng/mL; grp 5 - P $\geq 2.1\text{ng/mL}$. Pregnancy rates were then calculated for each group.

RESULTS AND CONCLUSIONS

The PRs of the IVF-ET cycles according to P concentration in different groups are shown in Table I. Pregnancy rates were considerably higher in P groups up to the level of 1.0ng/mL, but began to decline when P exceeded this level. There were 29 pregnancies in 67 cycles (43.2%) in patients with sera P levels of <1.0 (groups 1 and 2) versus only six pregnancies in 38 cycles (15.8%) in patients with a P level of 1.1 to 2.0ng/mL (groups 3 and 4) ($p=.007$ chi-square analysis). The viable PR was 38.8% for groups 1 and 2 (26/67) vs 13.1% for groups 3 and 4 (5/38) ($p<.007$ Fisher's exact test). Comparing total and viable PRs of groups

Table I Pregnancy Rate According to Progesterone Concentration At The Time Of hCG Administration

Group	Serum P (ng/mL)	No.	% total	Pregnancies (total)	PR (sac on ultrasound) (%)	Viable preg. (%)
1	<0.5	11	9.2	4	36.4	3 (27.3)
2	0.5-1.0	56	47	25	44.6	23 (41.1)
3	1.1-1.5	30	25.2	5	16.7	4 (13.3)
4	1.6-2.0	8	6.7	1	12.5	1 (12.5)
5	≥2.1	14	11.7	1	7.1	0 (0)
Total		119	100	36	30.2	31 (26.01)

1 and 2 combined vs group 3 alone (5/30 or 16.6% for total and 4/30 or 13.3% for viable) found $p < .05$ for both categories.

The incidence of premature luteinization with COH for IVF-ET is considerably reduced, but not eliminated through the use of GnRH α (4-9). Recently Mio et al. have provided data demonstrating that following clomiphene citrate (CC)-hMG COH for IVF, subtle P changes (measured by an RIA by Nippon Diagnostic Products) can occur during the follicular phase, without the rise in LH, and may be associated with a decreased fertility rate (10).

Thus, our data corroborate these other studies on the importance of measurement of sera P during the follicular phase when preparing for IVF-ET, even when a GnRH α is used. Furthermore, we show that the Amersham RIA can also be used effectively to demonstrate the adverse effect of higher sera P levels. However, with this assay, no adverse effect of P levels between 0.6 and 1.0 compared to ≤ 0.5 ng/mL could be determined. Each laboratory will have to evaluate its own results according to the assay kit employed. There are many centers that no longer measure serum P levels during COH for IVF-ET when GnRH agonists are used, because of the very low incidence of premature rise of LH. The importance of these data is to make the clinician aware that subtle rises in serum P may occur.

Confirmation of the importance of ultra-low levels of P are needed by other centers whether using RIA or non-isotopic immunoassay. It is not clear whether the subtle P rise in itself decreases the PR, e.g., by advancing the secretory effect on endometrium or is in some other way associated with reduction in fecundity. In addition, further work in the area of local ovarian factors may provide further insight to this phenomenon.

Thus, these data not only corroborate several other studies suggesting that a subtle rise of P before hCG, despite GnRH agonists may lower PRs and refutes the study by Edelstein (11), but emphasized the

importance of using an RIA assay for P with a low CV in the low range of P. One has to determine what cut-off for each specific assay delineates the high from low PR group. This may be especially true for non-isotopic methods for measuring P since many of these assays have never evaluated the CV of P in these low ranges.

REFERENCES

1. Check JH, Lurie D, Askari HA, Hoover L, Lauer C. The range of subtle rise in serum progesterone levels following controlled ovarian hyperstimulation associated with lower in vitro fertilization pregnancy rates is determined by the source of manufacturer. *Eur J Obstet Gynecol Reprod Biol* 52:205-209;1993.
2. Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil Steril* 55:563-566;1991.
3. Silverberg KM, Burns WN, Olive DL, Riehl RM, Schenken RS. Serum progesterone levels predict success of in vitro fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. *J Clin Endocrinol Metab* 73:797-803;1991.
4. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in vitro fertilization using buserelein and gonadotropins. *Lancet* 2:1284-1285;1984.
5. Neveu S, Hedon B, Bringer J, Chinchole JM, Arnal F, Humeau C, Cristol P, Viala JL. Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist and gonadotropins for in vitro fertilization. *Fertil Steril* 47:639-643;1987.
6. Meldrum DR, Wisot A, Hamilton F, Gutlay AL, Kempton W, Huynh D. Routine pituitary suppression with leuprolide before ovarian stimulation for oocyte retrieval. *Fertil Steril* 51:455-459;1989.
7. Stone BA, Serafini PC, Quinn K, Quinn P, Kerin JF, Marrs RP. Gonadotropin and estradiol levels during ovarian stimulation in women treated with leuprolide acetate. *Obstet Gynecol* 73:990-995;1989.
8. Chetkowski RJ, Kruse LR, Nass TE. Improved pregnancy outcome with the addition of leuprolide acetate to gonadotropins for in vitro fertilization. *Fertil Steril* 52:250-255;1989.
9. Check JH, Nowroozi K, Chase JS. Comparison of short versus long-term leuprolide acetate-human menopausal gonadotrophin hyperstimulation in in-vitro fertilization patients. *Hum Reprod* 7:31-34;1992.
10. Mio Y, Sekigima A, Iwabe T, Onohara Y, Harada T, Terakawa N. Subtle rise in serum progesterone during the follicular phase as a predictor of the outcome of in vitro fertilization. *Fertil Steril* 58:159-166;1992.
11. Edelstein MC, Seltman HG, Cos BJ, Robinson SM, Shaw RA, Muasher SJ. Progesterone levels on the day of human chorionic gonadotropin administration in cycles with gonadotropin-releasing hormone agonist suppression are not predictive of pregnancy outcome. *Fertil Steril* 54:853-857;1990.

MONDUZZI



EDITORE

VIA FERRARESE, 119/2
40128 BOLOGNA

TEL. (051) 370337 - FAX (051) 370529
TELEX 512654 MONDBO I